RELATIONSHIPS OF INORGANIC NITROGEN MEASUREMENTS TO THE PRESENCE OF FERTILIZER NITROGEN IN SOILS

by

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To Goube and Igue, whom

I love so much, this work

is dedicated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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INTRODUCTION

In any fertilizer program, nitrogen (N) is one element that is required in larger amounts for maximum food and fiber production. Except for leguminous plants that can fix elemental nitrogen, agricultural crops frequently need to be supplied this element for maximum growth. Nitrogen is largely present in the atmosphere in the elemental form (about 79.08 per cent by volume of the air). For N to become available to non-leguminous plants, it has to be fixed in ammonia (NH_3), ammonium (NH_4^+), or nitrate (NO_3^-) form. There are three known mechanisms of N fixation: symbiotic (legume+rhizobium); non-symbiotic (free-living organisms); and industrial N fixation. Among these mechanisms, for most crops and soils, industrial N fixation (chemical fertilizer N production) seems to be the ultimate solution for medium to high N problems, but it involves a lot of investment.

When N fertilizer is applied to soil, it enters several transformations and reactions. It can be absorbed by plant roots, assimilated by microorganisms, adsorbed on soil particles, leached deep into the profile or lost as gas. Due to this variety of fates among which only plant uptake is agronomically beneficial, N has to be added to keep an optimum level in soil for maximum yield, and to prevent surface and groundwater pollution.

Several researchers have studied N chemistry and transformations in soil in relation to source, time, rate, methods of application; soil and climatic conditions; and type of crop grown. The main reasons for these studies are to minimize the fertilizer N input, to maximize yield and recently to help to control water pollution.

In this study, the objectives were: (1) to relate changes in NH_4^+ and NO_3^- to the rates and times of N fertilizer application; (2) to evaluate the usefulness of measurements of changes in NH_4^+-N and NO_3^--N as a measure of the presence of fertilizer N; and (3) to study the eventuality of groundwater pollution.

LITERATURE REVIEW

High recovery of N and avoidance of pollution, have been given priorities in recent fertilizer N studies.

Fertilizer N applied prior to or during the period plants are growing results in maximum N uptake by the crop grown and thus higher yield. In corn and sorghum field experiments, the amounts of applied N recovered by the crops plus the residual N in the soil often amount to 70 to 90 per cent. The unaccounted 10 to 30 per cent is considered to be lost.

Nitrogen applied to soil, regardless of the source, must enter the soluble N pool as NO_3^--N or NH_4^+-N before it can be used by growing crops and microflora. Nitrogen not utilized by crops may be immobilized by microorganisms and subsequently become part of the organic matter; may remain in the inorganic form in the soil and be available for further crop use; or it may be lost from the soil profile.

Types of N Losses

Several types of losses of N from soils have been identified. Nitrogen immobilized by organisms is usually considered as lost even though it may become available to plants after the microorganisms die and are decomposed. Fixation of NH_4^+-ions between mineral lattices is considered a non-biological N immobilization. Alexander (1961) saw in this immobilization an agronomically important process because through it, microorganisms reduce the quantity of plant-available nutrients in soil. Immobilized N may be considered lost or slowly available.

The mobile NO_3^- form may be leached from the root zone if precipitation or irrigation exceeds the soil water holding capacity and the plant and evapotranspiration demands. Once this soluble N is leached below the root zone, for all practical purposes, no further use of the N is possible. This process is believed to be very crucial for sandy soils and in humid regions.

In addition to leaching, high soil water may contribute to poor aeration resulting in anaerobic conditions favorable for denitrification. Under conditions of low oxygen tension certain bacteria are capable of using NO_3^--N or NO_2^--N as a substitute for oxygen, reducing them to N_2O or elemental N. There is also a possibility of chemical denitrification under very acid conditions. All the gaseous forms of N are lost to the atmosphere. This biological or chemical reduction of N is one of the biggest loss mechanisms.

Volatilization losses of NH_3 can also be significant under some conditions if an NH_4^+ of NH_3 forming fertilizer is placed on or near the soil surface.

Some N losses by erosion may occur if fertilizer N is incorporated at a shallow depth in a sloping soil.

The magnitude of N losses by the different mechanisms depends upon several factors. Climatic and edaphic conditions have a marked effect. In addition to these two conditions, the source of fertilizer N and the time, rate and methods of application also may affect losses of N from fertilizers.

Factors Involved in N Losses

Immobilization. This is the assimilation of inorganic substances into the protoplasm of microorganisms. This type of immobilization is known as biological. Through this mechanism, microbes reduce the quantity of plant-available nutrients (Alexander 1961). When mineral N is added to cropped soils, a portion of it is assimilated by microorganisms and thereby converted into organic forms. The amounts so immobilized in cropped soils are greater

than in uncropped soils since more energy materials and more microorganisms are present in cropped soils (Allison 1966). In his study on fate of N, Allison (1966) observed that the amount of immobilized N depends on many factors such as crop species, rate and amount of growth, stage of growth of the plants and environmental conditions in soil. The immobilized N may be considered as temporarily lost, but it may become slowly available to further crops. This depletion of N is a function of number and type of microorganisms involved, and environmental conditions (Tisdale and Nelson 1975). Ammonium salts have a greater tendency to become assimilated than do other N forms with most bacteria, actinomycetes and fungi (Alexander, 1961; Smirnov, 1968). Immobilization of inorganic N may be detrimental to plants since they are poor competitors with microflora at low N level; but on the other hand, it may be beneficial in the autumn of the year in temperate regions by reducing leaching of water soluble N.

Another type of immobilization of N in soil beside the biological one, is known as fixation of NH_4^+-ions between the lattices of expanding types of minerals. Shaw (1962) found that NH_4^+-N was not leached from the surface layer of either light or heavy textured soils in his study of mineral N loss from soil.

Biological immobilization is normally of greater consequence than NH_4^+ fixation, so most of the N remaining in the soil after crop removal is in the organic forms.

Leaching is the mechanism through which the more soluble NO_3^--N is removed from the root zone by percolating water. Nitrate-nitrogen leaching is a channel of N loss in normal agricultural practices and it usually occurs in the fall and spring months in temperate regions (Allison, 1966). Hahne et al. (1977) showed that appreciable amounts of NO_3^--N accumulated in the soil when the optimum rate of fertilizer application had been exceeded, and that

vertical NO_3^--N distribution depends on soil type, amounts of irrigation water and precipitation, and that irrigation water markedly reduced the accumulation of NO_3^--N.

Nitrate-nitrogen movement is not due to complete displacement of soil solution by irrigation or rainfall water, but there occurs a gradual dilution out of the top soil (Bartholomew, 1965). Leaching of soluble N is more pronounced in early fall before soil is frozen and in the spring when there is little vegetative cover on the soil. Nitrate being the form subject to leaching, factors which influence the amount of nitrification may affect the extent of leaching. Nitrification is affected by the amount of NH_4^+-N present; oxygen supply (since the organisms involved, nitrosomonas and nitrobacter are aerobes); soil acidity; moisture content of the soil; seasonal effects; temperature and type of crops grown.

In temperate regions, NO_3^- formation is generally most rapid in spring and autumn, and slowest during summer and winter (Alexander, 1961). Muir et al. (1976) observed that leaching of NO_3^- to the water table was apparent in most valley and upland sandy soils receiving irrigation water; and alfalfa with its deep rooting system was an effective scavenger of inorganic N that may have accumulated under prior annual crops. Soil type, climate, grass-legume combination and grass pasture effects on leaching were studied by Theron et al. (1973). They found that grassland soil was not an important contributor to pollution in semiarid regions. Olsen et al. (1970) showed that NO_3^- in the profile was related to the rate of N application and the frequency of corn in rotations. Allison (1966) stated that climatic factors such as rainfall distribution and duration, and evapotranspiration are very important in considering NO_3^--N movement. Downward movement of NO_3^--N in light sandy soils is more pronounced than in heavy textured clay soils (Shaw, 1962). In the same study, Shaw observed also that NH_4^+-N is not leached in either soil.

Denitrification. If NO_3^--N is not utilized by plants, immobilized by microflora, or leached below the root zone, some of it may be lost by denitrification. This is a process through which NO_3^--N or NO_2^--N is reduced biologically or chemically to N_2O, NO, or elemental N and lost to the atmosphere (Glossary of Soil Science Terms, Soil Science Society of America, 1978). The process is carried out by facultatively anaerobic organisms.

Owens (1960) studied N transformation in soil using a lysimeter and found that 15 to 24 per cent of the applied N was recovered by plants, 38 per cent remained in the soil, 5 to 20 per cent was lost by leaching and 33 per cent by denitrification at the end of the second year of the experiment. Allison (1966) in his study of the fate of N, concluded that in fine textured soils, factors that are conducive to a high level of biological activity are the ones that are likely to result in lowering of dissolved oxygen in soil solution, which may or may not bring about NO_3^--N reduction. The high microbial activity leads to higher respiration rates, thus more carbon dioxide is evolved creating an anaerobic condition suitable to denitrification if NO_3^--N, food supply and the organisms are present. Chen et al. (1976) found that 37 per cent of added NO_3^--N fertilizer was in the organic and NH_4^+-N fraction, while 63 per cent was likely lost through denitrification, in their lake sediment study. Chemical decomposition of nitrite was studied by Forster et al. (1976) who observed that decomposition rate increased markedly with decrease in pH and proceeded rapidly even at low NO_2^- concentration. Studying the accumulation of NO_3^- in the soil profile, Jolley et al. (1977) showed that in addition to leaching, denitrification is one of the main causes of N losses. Mann et al. (1972) concluded that the addition of sulfur to a low organic matter soil increased the denitrification rate when Thiobacillus denitrificans was the organism involved.

Denitrification, carried out by heterotrophic facultative anaerobes, is affected by the energy supply (organic matter), oxygen concentration, NO_3^- content and other environmental conditions of the soil. Owens (1960) found that 27 to 39 per cent of the applied N was assumed to be lost by denitrification. Wilklander et al. (1977) in a study of NO_2^--N and NO_3^--N observed that there were considerable amounts of NO_3^--N in the upper layer of the profile receiving a high rate of fertilizer N in fall, but the next spring the content was lower. In this investigation they concluded that the seasonal difference was due to denitrification and other factors.

Heavy irrigation or rainfall may create anaerobic conditions favorable to denitrification by filling up the soil pore spaces.

Volatilization. In addition to the gaseous losses of N as N_2O, NO or molecular N by denitrification, there may be another gaseous loss in the form of NH_3. It is now well established that NH_3 may be volatilized from soils under certain conditions to the extent of 20 to 30 per cent of that added (Allison, 1955). The NH_3 volatilization is likely to occur when NH_3 or NH_4^+-salts are applied on or near the surface of alkaline soils. Morrison et al. (1977), in a urea $[CO(NH_2)_2]$ fertilizer study found that an estimated 30 per cent of applied N was lost by volatilization. Another very important study was conducted by Carter et al. (1961) on the effects of $(NH_4)_2SO_4$ application rates on gaseous losses of N. They found that losses of NH_3 during air-drying of samples were negligible in unlimed soil samples, but were as high as 15 per cent in the soil samples limed to pH 6.7 and receiving the largest amount of $(NH_4)_2SO_4$. This showed that NH_3 volatilization can also occur when NH_3 or NH_4^+-salts are applied to calcareous soils.

Erosion. Two mechanisms of erosion are chiefly involved, wind erosion and water erosion. Wind erosion operates largely on dry and unprotected soils,

and water erosion occurs on sloping lands. Power (1968) stated that soil erosion may result in serious losses of organic N and exchangeable inorganic N.

In unfavorable years and where proper control measures are not used, erosion may remove nearly as much N as is removed in the harvested crop (Bartholomew, 1965). The importance of losses depends on the erodability of the soil, the mechanisms involved and the management practices used.

Schuman et al. (1973) showed that N losses associated with sediments in the runoff accounted for 92 per cent of the total N loss from contour-planted corn watersheds. They also pointed out that runoff losses of N were usually highest at the beginning of the cropping season and decreased progressively throughout the year, reflecting a seasonal effect. In the same study, they observed that the conservation practice of level-terraced corn or pasture was very effective in reducing N losses by runoff.

The processes mentioned above are the most important mechanisms of N losses in soil, and some of the factors governing them. The control of these mechanisms, to a certain degree, governs the efficiency of N fertilizers and the extent of water pollution by excessive NO_3^--N.

It was noted that one of the main channels of N loss is leaching in humid areas and sandy soils. This mechanism should be viewed from two angles: plant nutrition and water quality. As far as plant nutrition is concerned the N leached out of the reach of roots is lost. For it to be usable agronomically, deep rooted crops such as alfalfa might be grown. If the leached N is not intercepted by plant roots, immobilized by organisms or denitrified, it may reach the ground water and becomes a pollutant, thus a water quality hazard.

Attempts have been made to control the conversion of NH_4^+-N to NO_3^--N which is subject to leaching and denitrification, by nitrification inhibitor

treatment. Chemical compounds such as N-serve have been tried and have shown some effects, but their effectiveness is largely dependent on temperature, acidity, moisture and texture of the soil. Other factors that have shown effects on N-transformations and losses are rate, time, and methods of fertilizer application; and the form of N fertilizer used (Allison, 1966; Morrison et al., 1977; Carter et al., 1961; and Smirnov et al., 1968).

MATERIALS AND METHODS

Experimental Sites

Two experimental sites were selected to study the movement of nitrogen through soil profiles. One of the sites (North Agronomy farm) located in Riley County was cropped with irrigated corn using rate as the variable. The other site (Ashland Agronomy farm) located in Riley County was cropped with wheat and was not irrigated, and application times and rates were used as variables.

Soil type, soil test data and other pertinent information for each site are given in Tables 1 to 3.

Designs of the Experiments

Two types of designs were used, one on each site. In the irrigated corn experiment, a completely randomized blocks design with three replications was used. Treatments included two rates of ammonium sulfate $\left[(NH_4)_2SO_4\right]$ fertilizer enriched about 6 per cent with ^{15}N. Nitrogen was applied in the spring at the rates of 0, 50 and 150 kgN/ha annually. Figure 1 shows the plot layout for the irrigated corn experiment.

The wheat experiment was a randomized complete block design with four replications. Treatments included two rates and two times of $(NH_4)_2SO_4$ fertilizer enriched about 7.5 per cent with ^{15}N. The dates of N application were fall and spring at the rates of 0, 50 and 100 kgN/ha annually. A diagram of the plots is given in Figure 2.

Table 1. General information about operations on each site.

Site	Soil Type	Date of N Application			
		Fall	Spring	Crop	Planting rate
North Agronomy Farm (Riley County)	Ivan silt loam	--	5-14-76	Corn	25,000 plants/a
	Cumulic Hapludolls	--	5-5-77		
	Fine silty, mixed, mesic				
Ashland Agronomy Farm (Riley County)	Reading silt loam	10-15-75	3-14-76	Wheat	60 lbs/a
	Typic Argiudolls	9-21-76	3-3-77		
	Fine, mixed, mesic				

Table 2. Irrigated corn site initial soil test data 1976.

Depth cm	pH value	Available P ppm	Exchangeable K ppm	Inorganic N ppm	Total N %
0-10	5.1	31.00	424.50	42.9	0.14
10-20	5.3	15.30	232.00	23.94	0.13
20-30	5.5	14.00	172.50	16.97	0.13
30-50	5.6	12.00	171.50	15.74	0.12
50-70	5.8	9.50	178.50	15.43	0.09
70-90	5.9	8.00	196.50	13.14	0.07
90-120	6.1	7.00	235.00	9.06	0.06
120-150	7.1	3.50	392.00	4.26	0.04
150-180	7.3	12.00	362.50	3.81	0.03

Table 3. Wheat experiment site initial soil test data 1975.

Depth cm	pH value	Available P ppm	Exchangeable K ppm	Inorganic N ppm	Total N %
0-10	5.70	75.50	431.00	21.36	0.13
10-20	5.70	71.00	370.50	18.58	0.12
20-30	5.90	27.00	351.00	13.79	0.11
30-50	6.10	18.50	305.00	11.87	0.09
50-70	6.30	21.00	267.50	8.85	0.08
70-90	7.00	15.50	341.50	8.83	0.08
90-120	7.80	9.00	365.00	11.98	0.08
120-150	7.30	12.50	276.00	8.26	0.09
150-180	7.40	17.00	285.00	5.21	0.06

FIGURE 1.

IRRIGATED CORN ^{15}N EXPERIMENT

NORTH AGRONOMY FARM

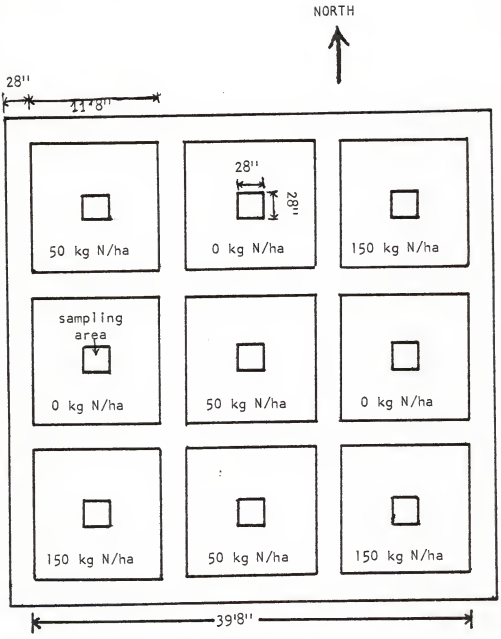
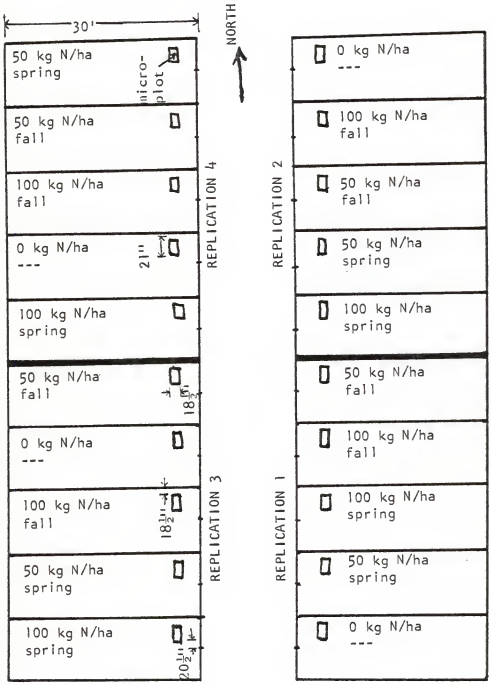


FIGURE 2. WHEAT ^{15}N EXPERIMENT

ASHLAND AGRONOMY FARM



Field Techniques

In the corn experiment plots were 11'8"x11'8" with the entire plot area being treated with ^{15}N. Sampling areas, 28"x28" in the center of each plot, were surrounded by metal rims to prevent movements of soil and water at the surface. Each plot was surrounded by a berm 28" in width as a further insurance against lateral soil and water movements. The entire plot area had a border of corn of four rows around it. The rows were spaced 28" apart with 5 rows in each plot and one row centered on the sampling area. The plant population was approximately 25,000 plants per acre. The corn was irrigated from an adjacent well with sprinklers when needed. The N treatments were applied each year in a water solution before planting corn. The fertilizer was mixed in the upper 10 cm. of soil after application. The experiment was carried out over a two year period. The first treatment was applied on May 14, 1976 and the second on May 5, 1977. Irrigation water was applied as necessary. Amounts of irrigation water applied and precipitation received each year are shown in Table 4.

In the wheat experiment, there were five plots per replication. The main plots, 10'x30', were treated with fertilizer grade $(NH_4)_2SO_4$. At one end of each plot a microplot for ^{15}N treatment was formed using 21"x18½" metal boxes pushed into soil to a depth of 4½ feet. The rows were spaced 7" apart and three rows crossed each microplot. The planting rate was about 60 lbs. per acre. Ammonium sulfate enriched with about 7.5 per cent ^{15}N was used as the N source in the microplot. The $(NH_4)_2SO_4$ was applied in a water solution. Fall applications were worked into the surface 10 cm. of soil. Spring applications were top dressed.

As in the corn experiment, the wheat study was carried out for a two year period. In each replication, there was one control, and four treated plots: 50 kgN/ha fall; 50 kgN/ha spring; 100 kgN/ha fall and 100 kgN/ha

Table 4. Amounts of precipitation and irrigation water received by plots in the irrigated corn experiment.

Water regime	Year 1976	1977	Total
Precipitation (inches)	13.62	43.79	57.41
Irrigation (inches)	26.00	11.40	37.40
Total	39.62	55.19	94.81

spring. The first year fall treatment was applied on October 15, 1975, and the second on September 21, 1976. The spring treatments were applied on March 14, 1976 for the first year and on March 3, 1977 for the second year. The amounts of precipitation received during the wheat experiment are shown in Table 5.

Sampling Procedures

Soil samples were collected from each experimental site prior to starting fertilizer treatments using a 1" Giddings soil sampler. With the wheat experiment, 16 individual samples from each replicate were composited. In the case of the corn experiment, 12 samples were taken from the entire area to give a single composite sample.

Soil samples were again collected at the end of the second crop from each sampling area. Plots were sampled on October 28, 1977 after corn was harvested and on July 6, 1977 for the wheat experiment.

In each case samples were taken to a depth of 180 cm at 10 cm intervals from 0-30 cm; at 20 cm intervals from 30-90 cm; and at 30 cm intervals from 90-180 cm.

With the wheat experiment four cores from each microplot were composited for the three surface layers and single cores were used for deeper depths. Samples from the corn experiment consisted of composites of 20 cores from the sampling areas for the surface 30 cm and four cores for the deeper depths. A rainy period following sampling of the 0-30 cm layers of treated plots with irrigated corn delayed additional sampling, so that 0-30 cm layers of untreated plots and deeper samples for all plots were sampled about one month later than the 0-30 cm layers of treated plots.

Table 5. Precipitation amounts received during the experimental period for wheat.

Periods	Fall Treatments	Spring Treatments
10-15-75 to 12-31-75	5.30	---
1-1-76 to 5-14-76	2.06	---
5-15-76 to 12-31-76	21.53	21.53
1-1-77 to 7-6-77	24.97	24.97
Total	53.86	46.50

The bags containing soil samples were placed in an oven heated at 50°C to dry. After drying, they were ground to pass an 80-mesh sieve and stored in air-tight glass bottles.

Laboratory Procedures

Exchangeable K, available P, available N and pH were run on initial soil samples by the Kansas State University soil testing laboratory.

NH_4^+-N and NO_3^--N analysis. A 15 gm soil sample was weighed, put into a 250 ml wide-mouth bottle, and 150 ml of 2M KCl were added. The bottle was stoppered and shaken for about one hour on a mechanical shaker. The soil-KCl suspension was allowed to settle until the supernant liquid was clear (about 30 min.). A 100 ml aliquot of the soil extract was put into a 250 ml distillation flask. A 125 ml Erlenmeyer flask marked to indicate 35 and 65 ml, and containing 5 ml boric acid indicator solution was placed under the condenser of a steam-distillation apparatus. The distillation flask was attached to the distillation apparatus. By the side arm, using a funnel, 1 gm of MgO was added and the stopcock of the steam-bypass was closed. When the distillate volume reached the 65 ml mark on the receiver flask, distillation was stopped by opening the steam-bypass stopcock. For the ^{15}N samples the distillation flask was removed after distillate reached the 35 ml mark, and a 100 ml distillation flask containing 15 ml of 95% ethanol was attached to the distillation apparatus. Distillation was allowed to proceed until the distillate reached the 65 ml mark. In both cases the distillation was stopped and distillation flask removed, the condenser ends and the steam outlets were cleaned with distilled water. The distillate was titrated with standard 0.0049N H_2SO_4 for NH_4^+-N. The end-point color change was from green to a permanent faint pink.

After removal of NH_4^+-N, another 125 ml Erlenmeyer flask marked to show 35 and 65 ml, and containing 5 ml boric acid-indicator solution was placed under the condenser. The distillation flask from which NH_4^+-N was removed was attached to the distillation apparatus and 1 gm of Devarda's alloy was added using a funnel. The steam-bypass stopcock was closed and the same procedure was followed as in the case of NH_4^+-N determination after addition of MgO, to determine $(NO_3^- + NO_2^-)$-N.

In both NH_4^+-N and $(NO_3^- + NO_2^-)$-N determinations, the samples were duplicated. Blanks were carried out the same way as samples. Parts per million of NH_4^+-N and $(NO_3^- + NO_2^-)$-N were then calculated by the formula.

$$\text{ppm N} = \frac{(\text{mls of } H_2SO_4 \text{ for sample} - \text{mls of } H_2SO_4 \text{ for blank})(\text{Normality } H_2SO_4)(0.014)(10^6)}{\text{Sample weight (gm)}}$$

^{15}N Determination. After titration, the duplicates were put into separate beakers and 0.3 ml of 1N H_2SO_4 were added. The beakers were set on a hot plate adjusted to give slow evaporation. When the volume of the beakers' solution reached about 5 ml, the duplicates were combined and heated until a final volume of around 5 ml was achieved. The same thing was done for NH_4^+-N and NO_3^--N separately. The final 5 ml concentrated solution was put in a numbered vial and stored in a refrigerator. When all the samples were analyzed and concentrated, the vials were sent to the University of Arizona, Tucson, for ^{15}N analyses by the mass spectrometer method. Percentage of fertilizer N recovered as NH_4^+-N and NO_3^--N and per cent NH_4^+-N and NO_3^--N from fertilizer were calculated using the following formulae.

(1) North farm soil samples--corn experiment

(a) for 50 kg N/ha treatments

$$\%\ \text{fertilizer N recovered} = \frac{(A\%\ ^{15}N - 0.3611)(\text{uncorrected titration})}{(\text{kg soil})(0.0245)}$$

% NH_4^+ or NO_3^--N in horizon from fertilizer=

$$\frac{\text{(\% fertilizer N recovered)}}{\text{(corrected titration) (kg soil)}} \quad (734)$$

where corrected titration=(uncorrected titration-blank)

(b) for 150 kg N/ha treatments

% fertilizer N recovered=(A% ^{15}N-0.3611)(uncorrected titration)(kg soil)(0.00815)

% NH_4^+ or NO_3^--N in horizon from fertilizer=

$$\frac{\text{(\% fertilizer N recovered)}}{\text{(corrected titration) (kg soil)}} \quad (2202)$$

(2) Ashland farm soil samples--wheat experiment

(a) for 50 kg N/ha treatments

% fertilizer N recovered=(A% ^{15}N-0.3598)(uncorrected titration)(kg soil)(0.0376)

% NH_4^+ or NO_3^--N in horizon from fertilizer=

$$\frac{\text{(\% fertilizer N recovered)}(364.8)}{\text{(corrected titration)(kg soil)}}$$

(b) for 100 kg N/ha treatments

% fertilizer N recovered=

(A% ^{15}N-0.3598)(uncorrected titration)(kg soil)(0.0188)

% NH_4^+ or NO_3^--N in horizon from fertilizer=

$$\frac{\text{(\% fertilizer N recovered)}(729.4)}{\text{(corrected titration)(kg soil)}}$$

Statistical Methods

NH_4^+-N and NO_3^--N were evaluated using the Snedocor and Cochran analysis of variance (AOV) procedures.

RESULTS AND DISCUSSION

Most of the studies reviewed by the author have used NO_3^--N measurements to assess movement of N fertilizers. The main objective of this study was to measure changes of inorganic N forms (NH_4^+-N and NO_3^--N) in the profile and compare them with movements of fertilizer N determined by ^{15}N-tracer measurements.

Irrigated Corn Experiment

The NH_4^+-N concentrations in the profiles treated with 0, 50 and 150 kg N/ha after two years are represented in Figure 3. The results show NH_4^+-N throughout the profiles and no accumulation peaks are observed. The higher NH_4^+-N level for the control in the surface may have been due to later sampling of control plots. The analysis of variance of NH_4^+-N in Table 6 does not show any significant effect of the application rates used at the 5% α-level, but there is a difference with depth and depth x amount within each treatment at the 1% α-level. The surface 30 cm for all treatments had higher NH_4^+-N, but this decreased with depth in the profile.

Table 7 compares NH_4^+-N concentrations in the profile before treatment with those after two years of treatments. Concentrations of NH_4^+-N were lower in the surface horizons after two years of cropping, but were greater at lower depths. Since NH_4^+-N is consumed by immobilizing microorganisms and released by ammonifying organisms, observed differences may reflect the status of these processes at the particular times samples were taken. The presence of NH_4^+-N throughout the profiles may be explained by mineralization of organic N since the total N is fairly high throughout the profiles.

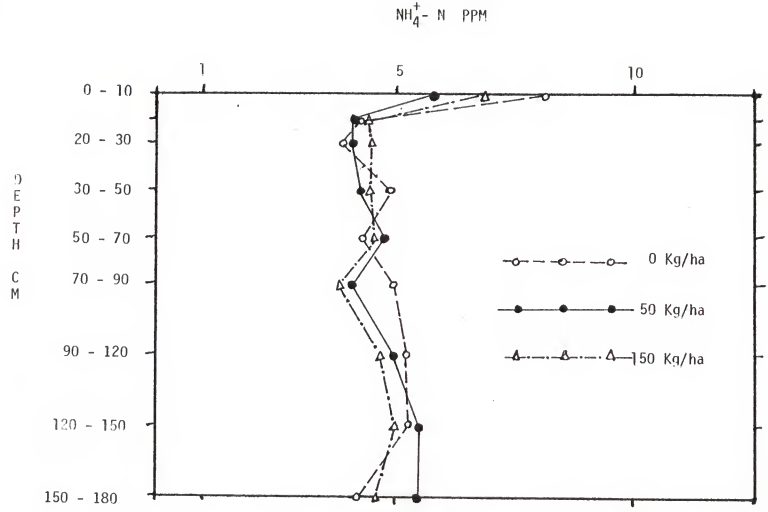


Figure 3. NH_4^+ - N concentration in soil profiles after two years of fertilizer treatments with corn.

Table 6. Analysis of variance of NH_4^+-N contents of soils from experiment with irrigated corn.

Source of variation	df	Sum of squares	Mean of squares	F
Amount	2	.96	.48	.22
Error A	6	13.09	2.18	---
Depth	10	52.73	5.273	12.27**
Amount x Depth	20	18.67	.9x33	2.17**
Error B	60	25.78	.43	---

**Significant at 1% α-level.

Table 7. Concentrations of NH_4^+-N in soils before treatment and after two years of fertilizer N treatments on irrigated corn.

Depth cm	NH_4^+-N in soil, ppm			
	Before treatment	After two years of indicated treatment		
		0 kg N/ha	50 kg N/ha	150 kg N/ha
0-10	20.56	8.13	5.81	6.93
10-20	3.76	4.33	4.18	4.45
20-30	7.35	3.90	4.13	4.54
30-50	6.38	4.90	4.36	4.44
50-70	4.39	4.35	4.79	4.60
70-90	3.57	5.02	4.15	3.84
90-120	3.50	5.30	5.08	4.70
120-150	2.13	5.41	5.55	5.03
150-180	2.06	4.57	5.51	4.69

The NO_3^--N concentrations for 0, 50 and 150 kg N/ha treatments are shown in Figure 4. The results reveal a gradual decrease in NO_3^--N concentration with depth in all profiles. The higher NO_3^--N content of the control in 0-10 cm layer is again probably due to a later sampling date for these plots during a period when conditions were favorable for nitrification of soil N. The analysis of variance in Table 8 does not show any significant difference between treatments applied, but depth and amount x depth show significance at 1% α-level.

The NO_3^--N content decreased from 12-16 ppm at the surface to less than 1 ppm at 180 cm. At deeper points in the profiles, the NO_3^--N concentrations became less than the NH_4^+-N concentrations for all treatments. This again might be due to immobilization of NO_3^--N by microorganisms and mineralization of organic N to produce NH_4^+-N. By comparing the NO_3^--N of the soil before treatment with the treatments after two years, in Table 9, it can be seen that the treatments have lowered concentrations of NO_3^--N at all depths. This may be explained by the crop removal, immobilization, denitrification and some leaching of NO_3^--N. The low concentrations of NO_3^--N and its gradual decrease show there is no accumulation at any depth and the risk of ground water pollution is small.

Since the fertilizer N applied to these plots was tagged with ^{15}N, it was possible to determine the quantities of fertilizer N in the NH_4^+ and NO_3^- forms for those samples which contained sufficient N for mass spectrometer analyses. Results of these determinations are shown in Table 10. Only the surface 30 cm contained enough inorganic N for analysis. From 4.52 to 21.33% of the NH_4^+-N and 3.39 to 17.59% of the NO_3^--N in the soil was from the fertilizer. The balance of the inorganic N present was that which was present before the fertilizer was added or was produced from mineralization of organic N.

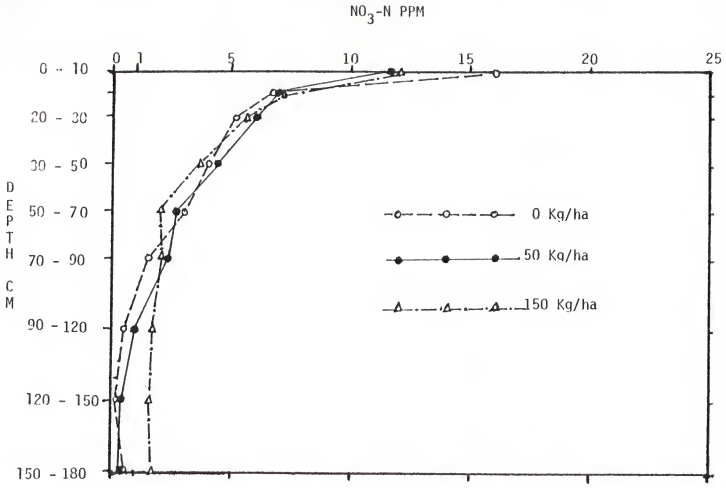


Figure 4. NO_3^- - N Concentration in soil profiles after two years of fertilizer treatments with corn.

Table 8. Analysis of variance of NO_3^--N contents of soils from experiment with irrigated corn.

Source of variation	df	Sum of squares	Mean of squares	F
Amount	2	1.60	.80	.17
Error A	6	29.00	4.83	---
Depth	10	1272.93	127.29	137.41**
Amount x Depth	20	47.77	2.395	2.58**
Error B	60	55.58	.926	---

**Significant at 1% α-level.

Table 9. Concentrations of NO_3^--N in soils before treatment and after two years of fertilizer N treatments on irrigated corn.

Depth cm	NO_3^--N in soil, ppm			
	Before treatment	After two years of indicated treatment		
		0 kg N/ha	50 kg N/ha	150 kg N/ha
0-10	22.34	16.02	11.69	12.11
10-20	14.18	6.76	6.81	7.08
20-30	9.62	5.22	6.07	5.69
30-50	9.36	4.09	4.46	3.77
50-70	11.04	3.05	2.80	2.09
70-90	9.57	1.58	2.35	2.11
90-120	5.56	0.57	1.07	1.75
120-150	2.13	0.39	0.46	1.64
150-180	1.75	0.54	0.49	1.73

Table 10. Amounts of NH_4^+- and NO_3^--N in surface soil layers derived from fertilizer with the irrigated corn experiment.

Treatment	Soil layer	NH_4^+-N from fertilizer		NO_3^--N from fertilizer	
kg N/ha	cm	ppm	%	ppm	%
50	0-10	0.26±.04	4.52±.51	0.84±.22	7.29±.32
	10-20	---	---	0.27±.04	3.94±.06
	20-30	---	---	0.20±.04	3.39±.07
150	0-10	1.00±.15	14.53±2.32	1.96±.63	17.59±1.61
	10-20	0.96±.13	21.33±1.32	0.78	11.55±1.14
	20-30	---	---	0.57±.09	11.22±.32

Table 11. Amounts of NH_4^+-N and NO_3^--N from fertilizer in 0-10 cm layers of treated plots for irrigated corn.

Treatment	ppm N from fertilizer in 0-10 cm layer in indicated form		% of indicated form of N in 0-10 cm layer from fertilizer	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-
50 kg N/ha	0.26 b*	0.84 a	4.52 b	7.29 b
150 kg N/ha	1.00 a	1.96 a	14.53 a	17.50 a

*Means followed by the same letter in each column are not significantly different as determined by Duncan's multiple range test at 5% α-level.

Larger quantities of both NH_4^+- and NO_3^--N from fertilizer were present with 150 kg N than 50 kg N treatments. In the case of NH_4^+-N, this difference corresponded with differences in total NH_4^+-N present (Figure 3). With NO_3^--N, there was little difference between total amounts present for the two treatments (Figure 4), but surface soils from the 150 kg N treatment had two to three times as much NO_3^--N from the fertilizer as those from the 50 kg N treatment. These results would indicate measurements of inorganic N are not a very sensitive method for determining the presence of N originating from fertilizer.

Other data from these same plots (Olson, R. V., personal communication) indicate when total N was considered, some fertilizer N had moved to a depth of 120 cm with the 50 kg N treatment and to 240 cm with the 150 kg N treatment. This fertilizer N is not reflected in differences between amounts of inorganic N in treated and untreated plots in this experiment, again showing such measurements are not sensitive indicators of fertilizer N movements.

Most of the NH_4^+-N and NO_3^--N from the fertilizer being in the 0-10 cm layer, an analysis of variance for these forms in this layer was made. Table 11 shows mean values and results of the Duncan multiple range test for ppm of inorganic N and per cent inorganic N from fertilizer. NH_4^+-N amounts from the fertilizer were significantly higher for the 150 kg N/ha treatments than the 50 kg N/ha. There was no significant difference in NO_3^--N amounts from the fertilizer due to treatments. Table 11 shows also that 150 kg N/ha treatments had significantly greater per cent NH_4^+-N and NO_3^--N from the fertilizer than 50 kg N/ha in the 0-10 cm layer.

Wheat Experiment

The NH_4^+-N concentrations in the profiles of treated plots after two years are represented in Figure 5. The curves reveal the existence of NH_4^+-N

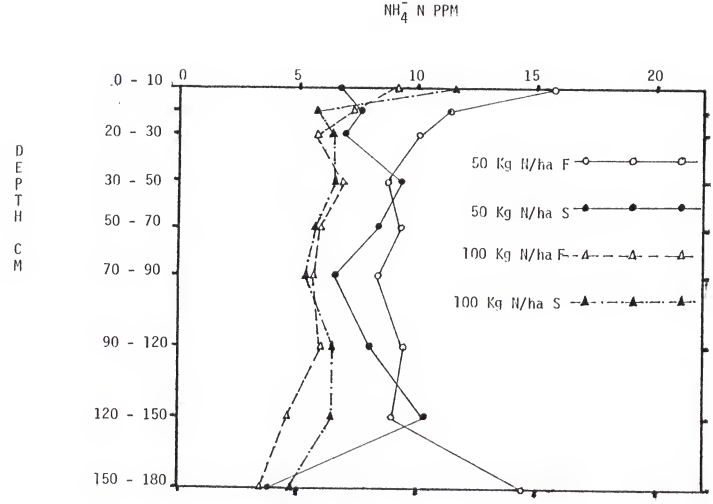


Figure 5. NH_4^+- N Concentration in soil profiles after two years of fertilizer treatments with wheat.

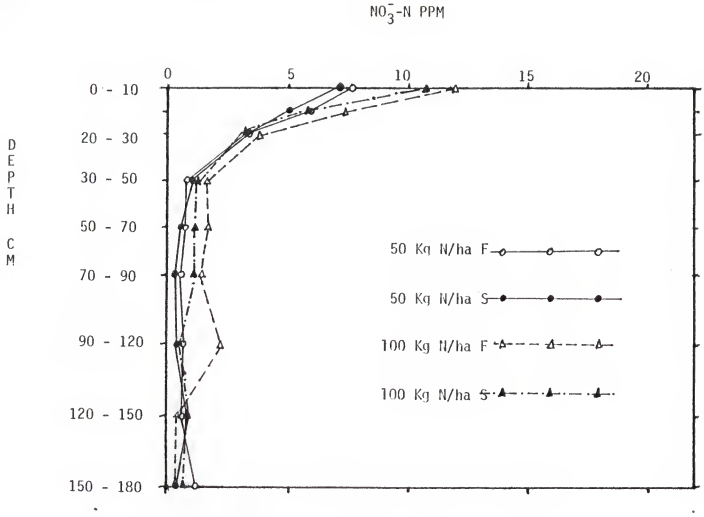


Figure 6. NO_3^--N Concentration in soil profiles after two years of fertilizer treatments with wheat.

throughout the profile following a chromatogramic pattern. The analysis of variance of NH_4^+-N in Table 12 does not show any significant effect of the treatments applied at 5% α-level.

Table 13 gives the NH_4^+-N contents in the profiles before treatment and after two years of treatments. All the treated plots had lower NH_4^+-N than before treatment. This decrease could be explained by crop removal and immobilization by microflora. Since NH_4^+-N levels decreased, comparisons of NH_4^+-N levels following treatment with initial levels are not a good means of detecting the presence of fertilizer N.

The NO_3^--N contents for the treatments after two years are represented in Figure 6. The results show a gradual decrease in NO_3^--N contents with depth for all treatments, except the 100 kg N/ha fall treatment which shows a small accumulation peak in the 90-120 cm layer. The analysis of variance in Table 14 shows no significant effect of rate and time of application at the 5% α-level. However, depth and depth x amount interaction had a significant effect on distribution of NO_3^--N in the profiles. Conversely to the NH_4^+-N contents, the NO_3^--N contents of the 100 kg N/ha treatments were higher than that of 50 kg N/ha treatments. This might explain the low NH_4^+-N level of the 100 kg N/ha treatments due to nitrification. The concentrations of NO_3^--N at lower depths are less than 2 ppm for all treatments indicating fertilizer treatments did not influence deep soil nitrate levels. In Table 15, it can be seen that after two years NO_3^--N in treated plots was higher in upper 30 cm layers than before treatment. At depths below 30 cm treated plots showed a decrease in NO_3^--N. This would indicate fertilizer N was contributing to the NO_3^--N supply to a depth of only 30 cm. At lower depths, crop removal, leaching, and denitrification may all have contributed to depletion of NO_3^--N. The low levels of NO_3^--N would indicate little probability of pollution of ground water with NO_3^--N.

Table 12. Analysis of variance of NH_4^+-N contents of soils from wheat experiment.

Source of Variation	df	Sum of Squares	Mean of Squares	F
Amount (A)	1	284.68	284.68	4.59
Rep	3	293.84	97.95	---
Time (T)	1	63.03	63.03	1.02
AxT	1	120.10		1.94
Error A	9	557.65	61.96	---
Depth (D)	8	205.07	25.63	1.47
DxA	8	47.05	5.88	0.34
DxT	8	115.27	14.41	0.82
AxTxD	8	175.55	21.94	1.25
Error B	96	1679.02	17.49	---

Table 13. NH_4^+-N concentrations in soil before treatments and after two years of fertilizer N treatments with wheat.

Depth cm	NH_4^+-N in soil, ppm				
		After two years of indicated treatment			
	Before treatment	50 kg/ha fall	50 kg/ha spring	100 kg/ha fall	100 kg/ha spring
0-10	17.03	15.61	6.81	9.15	11.57
10-20	13.90	11.27	7.65	7.28	5.72
20-30	10.69	10.04	6.95	5.76	6.46
30-50	10.04	8.75	9.34	6.87	6.57
50-70	7.53	9.30	8.36	5.90	5.74
70-90	7.93	8.35	6.58	5.58	5.29
90-120	10.61	9.48	8.05	5.96	6.48
120-150	6.95	8.97	10.36	4.51	6.39
150-180	4.15	14.41	3.73	3.43	4.73

Table 14. Analysis of variance of NO_3^--N contents of soils from wheat experiment.

Source of Variation	df	Sum of Squares	Mean of Squares	F
Amount (A)	1	27.34	27.34	4.99
Rep	3	11.99	4.00	---
Time (T)	1	8.32	8.32	1.52
AxT	1	1.50	1.50	0.27
Error A	9	49.27	5.47	---
Depth (D)	8	1229.94	153.74	65.70*
DxA	8	49.70	6.21	2.65*
DxT	8	5.73	0.72	0.31
AxDxT	8	3.94	0.49	0.21
Error B	96	224.32	2.34	---

*Significant at 5% α-level.

As with the corn experiment, the fertilizer N applied to these treated plots was tagged with ^{15}N. This made it possible to determine the quantities of fertilizer N in the NH_4^+ and NO_3^--N forms for the samples containing sufficient N for mass spectrometer analyses. The results of these analyses and calculations are given in Table 16 for the fall treatments and in Table 17 for the spring treatments. Only the surface 90 cm for NH_4^+-N and 20 cm for NO_3^--N contained enough inorganic N for mass spectrographic analysis.

While total NH_4^+-N levels were high throughout the profile for all treatments and showed little change with depths (Table 12), NH_4^+-N from fertilizer was confined largely to the 0-10 cm layer with lesser amounts between 10 and 30 cm and only insignificant amounts below 30 cm. Percentages of NH_4^+-N present coming from fertilizer ranged from 8.9 to 16.5 in the 0-10 cm layer and decreased to as little as 0.01% at 70-90 cm. Percentages of NO_3^--N present coming from fertilizer were also small, being from 5.8 to 16 for surface 20 cm layer to not detectable below 20 cm. The fact that only small amounts of NH_4^+-N and NO_3^--N present came from fertilizer may explain why measurements of total NH_4^+-N and NO_3^-N did not reflect the presence of fertilizer N.

Since most of the NH_4^+-N and NO_3^--N from the fertilizer was in the 0-10 cm layer, an analysis of variance for inorganic N contents in this layer was made. Table 18 shows mean values and results of the Duncan multiple range test for ppm of inorganic N and % inorganic N from the fertiiizer. There was no significant difference in ppm NH_4^+-N from fertilizer due to treatments. NO_3^--N amounts from the fertilizer, however, were significantly greater for 100 kg N treatments than 50 kg N. Within the same application rate, the application time did not have any significant effect on either NH_4^+-N or NO_3^--N from fertilizer. The significance in the NO_3^--N contents from the fertilizer found in the 0-10 cm layer corresponded to the total NO_3^--N differences (Figure 6) even though the later differences were not statistically significant.

Table 15. NO_3^--N concentrations in soil before treatments and after two years of fertilizer N treatments with wheat.

	NO_3^--N in soil, ppm				
		After two years of indicated treatment			
Depth cm	Before treatment	50 kg N/ha fall	50 kg N/ha spring	100 kg N/ha fall	100 kg N/ha spring
0-10	4.33	7.73	7.15	12.00	10.80
10-20	4.67	5.82	5.07	7.41	5.81
20-30	3.09	3.24	3.45	3.87	3.26
30-50	1.82	0.87	0.93	1.65	1.13
50-70	1.31	0.76	0.64	1.71	1.11
70-90	0.90	0.59	0.34	1.39	1.10
90-120	1.37	0.62	0.42	2.21	0.55
120-150	1.31	0.69	0.72	0.49	0.73
150-180	1.05	1.19	0.31	0.47	0.54

Table 16. Amounts of NH_4^+- and NO_3^--N in soil derived from fertilizer for fall treatments with the wheat experiment.

Treatment	Soil layer	NH_4^+-N from fertilizer		NO_3^--N from fertilizer	
	cm	ppm	%	ppm	%
50 kg N/ha, fall	0-10	1.78±.68	11.4±2.73	0.92±.39	11.90±3.26
	10-20	0.33±.06	2.96±.92	0.24±.07	4.21±.52
	20-30	0.14±.05	1.39±.36	---	---
	30-50	0.02±.005	0.23±.090	---	---
	50-70	0.02±.005	0.21±.10	---	---
	70-90	0.01±.08	0.16±.14	---	---
100 kg N/ha, fall	0-10	1.51±.51	16.51±7.00	1.90±.44	15.82±3.55
	10-20	0.41±.22	5.51±2.76	0.43±.08	5.80±1.59
	20-30	0.16±.08	2.86±2.55	---	---
	30-50	0.03±.01	0.46±.20	---	---
	50-70	0.03±.01	0.46±.20	---	---
	70-90	0.02±.006	0.43±.02	---	---

Table 17. Amounts of NH_4^+- and NO_3^--N in soil derived from fertilizer for spring treatments with the wheat experiment.

Treatment	Soil layer cm	NH_4^+-N from fertilizer ppm	NH_4^+-N from fertilizer %	NO_3^--N from fertilizer ppm	NO_3^--N from fertilizer %
50 kg N/ha, spring	0-10	0.60±.33	8.91±4.76	0.78±.24	10.97±5.98
	10-20	0.19±.19	2.51±1.99	---	---
	20-30	0.12±.06	1.68±.56	---	---
	30-50	0.02±.005	0.27±.11	---	---
	50-70	0.03±.008	0.32±.24	---	---
	70-90	0.03±.02	0.46±.38	---	---
100 kg N/ha, spring	0-10	2.26±1.93	19.5±4.16	1.73±.16	16.00±1.53
	10-20	0.30±.07	5.23±.79	0.74±.21	12.70±1.50
	20-30	0.18±.03	2.75±.37	---	---
	30-50	0.06±.04	0.96±.92	---	---
	50-70	0.06±.03	0.99±.82	---	---
	70-90	0.18±.24	3.38±4.37	---	---

Table 18. Amounts of NH_4^+-N and NO_3^--N from fertilizer in 0-10 cm layers of treated plots for wheat experiment.

	ppm N from fertilizer in 0-10 cm layer in indicated form		% of indicated form of N in 0-10 cm layer from fertilizer	
Treatment	NH_4^+	NO_3^-	NH_4^+	NO_3^-
50 kg N/ha, fall	1.58a*	0.92 b	11.40 bc	11.9 ab
50 kg N/ha, spring	0.60a	0.78 b	8.92 c	10.97 b
100 kg N/ha, fall	1.44a	1.71a	16.51ab	15.87a
100 kg N/ha, spring	2.24a	1.72a	19.50a	16.0 a

*Means followed by the same letter in each column are not significantly different as determined by Duncan's multiple range test at 5% α-level.

Table 17 shows also that application times had no significant effect on percentages of the NH_4^+-N or NO_3^--N which originated from the fertilizer. Percentages of both forms of N were higher for 100 kg N rates than for the 50 kg N/ha applied in spring, and 100 kg N applied in spring gave a significantly higher percentage of NH_4^+-N from fertilizer than 50 kg N in the fall. The higher rate of fertilizer N thus resulted in a larger portion of the inorganic N coming from fertilizer.

SUMMARY AND CONCLUSIONS

Times and rates of fertilizer N application in the two experiments did not show a significant effect, at 5% -level on the NH_4^+-N and NO_3^--N contents. In both experiments it was found that NH_4^+-N concentrations were greater than the NO_3^--N in all treatments after two years excepting in the 0-10 cm layers. In irrigated corn soils more NO_3^--N was found than in the wheat experiment, whereas more NH_4^+-N was found under wheat. Most of the total NO_3^--N existed in the surface 30 cm layer in all treatments for the two locations. The NH_4^+-N distribution in the profiles followed a chromatogramic pattern. Despite the non-significance of rates or times of fertilizer N application, 100 kg N rates in the wheat experiment had larger amounts of NO_3^--N in the profile, but lower NH_4^+-N than the 50 kg N rates.

The analysis of ^{15}N data revealed two to three times as much N from fertilizer in the NO_3^--N form and three times as much NH_4^+-N in the irrigated corn experiment at the 150 kg N rate as at the 50 kg N rate. In the wheat experiment the ^{15}N analysis indicated twice as much NO_3^--N from fertilizer in the 100 kg N rate as in the 50 kg N rate but no significant differences in NH_4^+-N from fertilizer.

In both experiments, ^{15}N data showed the presence of inorganic forms of N in the soil in substantial quantities, especially in surface layers. Measurements of total amounts of NH_4^+ and NO_3^- forms of N, however, did not reflect the presence of fertilizer N. This is probably because most of the inorganic N present originated from indigenous soil N rather than fertilizer N. It would appear from results of these two experiments that measurements of inorganic N are not a very sensitive means of detecting the presence of fertilizer

N, at least when moderate application rates of fertilizer N are involved.

The ground water pollution possibilities were small due to the low NO_3^--N concentrations deeper in the profiles of all treatments at both locations. But since there was an indication of more NO_3^--N in the irrigated corn soils than the wheat experiment, the risks of pollution might be expected to be higher under irrigated corn cropping than wheat. The same observation was made by ^{15}N analysis which showed higher NO_3^--N from fertilizer under corn compared to wheat. Higher rates under corn yielded more NO_3^--N from fertilizer showing again the risks of pollution if rate were increased.

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RELATIONSHIPS OF INORGANIC NITROGEN MEASUREMENTS TO THE PRESENCE OF FERTILIZER NITROGEN IN SOILS

by

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A two year experiment was conducted to assess fertilizer N movements in the soil profile and the possibility of groundwater pollution, using NH_4^+- and NO_3^--N measurements on two sites in Kansas (North Agronomy farm and Ashland farm). Irrigated corn was grown at one location and wheat at the other. Ammonium sulfate $\left[(NH_4)_2SO_4\right]$ enriched with ^{15}N tracer was used as the N source on both locations. The rates of application were 0, 50 and 150 kg N/ha annually for the irrigated corn and 0, 50 and 100 kg N/ha annually for the wheat experiment. In the wheat experiment there were two application times (fall and spring) used to study the application time effects on inorganic forms of N and their movement in the profile.

In both experiments, the treatments applied did not show any significant effect on NH_4^+- and NO_3^--N concentrations after the two years.

Soils from the wheat experiment had lower NO_3^--N concentrations as compared to soils under irrigated corn.

The ^{15}N analysis of the irrigated corn soils showed two to three times more N from fertilizer as NO_3^--N in the surface 30 cm of soils from the plots that received 150 kg N as compared with those that received the 50 kg N rate. With the wheat experiment, surface layers of 100 kg N/ha treatments contained about twice as much NO_3^--N from fertilizer as 50 kg N/ha treatments. Amounts of NH_4^+-N from the fertilizer were greater with the higher rates of N with irrigated corn, but not with wheat.

There were no significant differences in amounts of total inorganic N among treatments even though large differences in amounts of NO_3^--N from fertilizer were found. These results show, at least when moderate fertilizer N

application rates are concerned, that measurements of inorganic soil N do not provide a sensitive means of detecting the presence of fertilizer N.

Nitrate-N levels in subsoils for both experiments were low for all treatments. This would indicate little likelihood of pollution of groundwater supplies with NO_3^--N.